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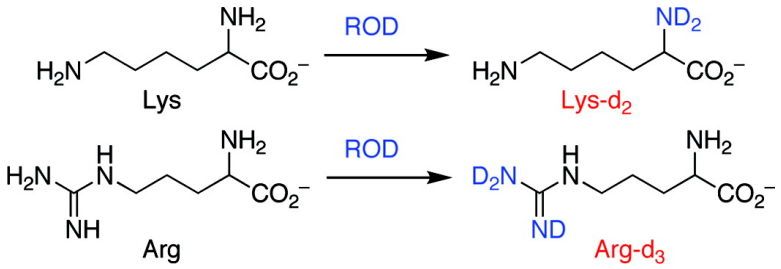
J. Am. Chem. Soc., **2008**, 130 (1), 8-9 • DOI: 10.1021/ja076599l



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Published on Web 12/08/2007

Hydrogen−Deuterium Exchange and Selective Labeling of Deprotonated Amino Acids and Peptides in the Gas Phase

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Hydrogen atoms attached to oxygen, nitrogen, and sulfur can be replaced with deuterium upon reacting protio compounds with deuterium oxide or deuterated alcohols (ROD). The isotopic exchange of these labile hydrogens is an important tool for the determination of protein structures and the study of dynamic processes such as protein folding.[1] Isotopically labeled species also provide a valuable means for obtaining mechanistic information. We report herein on the H/D exchange of deprotonated amino acids and small peptides. To our surprise, site-specific labeling can be carried out on the M − H ions of arginine, lysine, and a variety of small peptides with select deuterated reagents. The replacement of only some of the labile hydrogens should prove useful in probing reaction mechanisms and the mobile nature of the proton. In addition, the sequence order is found to effect the extent of exchange.

The conjugate base of glycine undergoes two H/D exchanges with CF_3CH_2OD,[2] and molecular modeling indicates that this process takes place via a four-centered flip-flop process in which the O−D and N−H bonds interchange via a zwitterionic transition structure (see Figure S1 in the Supporting Information).[3] Given these results, it is not surprising that the M − H ions of Arg, Asn, Cys,[4] Glu, His, Pro, Thr, Trp, and Tyr undergo exchange of their α-amino hydrogens with CF_3CH_2OD. It is less apparent what will happen to the hydrogens attached to the side-chain heteroatoms, but they too are replaced with deuterium presumably by a variety of processes.[3−5] Lysine, however, is surprising in that only one of the two amino groups undergoes H/D exchange (Figure 1).[6] Given that the disappearance rate constant of the d_0 ion of Lys is similar to that of glycine ($k = (2.2 \pm 0.7) \times 10^{-11}$ and $(2.6 \pm 0.8) \times 10^{-11}$ cm^3 molecule^{-1} s^{-1}, respectively),[7] this suggests that it is the hydrogens on the α-amino group which are replaced rather than those on the side chain.

To test this inference, the reactivities of *N*(α)-methyllysine ($H_2N(CH_2)_4CH(NHMe)CO_2^-$) and *N*(ϵ)-methyllysine ($MeNH(CH_2)_4CH(NH_2)CO_2^-$) were explored. The former compound undergoes one H/D exchange, whereas the latter incorporates two deuteriums, thereby confirming that it is the α-amino group of lysine which undergoes exchange. Electrostatic interactions can account for this observation since the α-amino group is closer to the charged site. That is, a zwitterionic transition state or intermediate with a NH_3^+ group is expected to be more stable at the α-position than on the more distant side chain. This difference should diminish with more acidic exchange reagents since they lead to faster reactions (measured rate constants are given in Table S1). In accord with this expectation, all of the labile hydrogens in the M − H ion of Lys rapidly undergo exchange with d_6-phenol ($k = (1.1 \pm 0.3) \times 10^{-9}$ cm^3 molecule^{-1} s^{-1}), which is 12 kcal mol^{-1} more acidic than CF_3CH_2OH.[8]

Less acidic exchange reagents react more slowly, and $C_6H_5CH_2OD$ and CH_3CH_2OD were found to be unreactive with deprotonated glycine and other nonacidic side-chain-containing amino acids (i.e., $k \leq 1 \times 10^{-13}$ cm^3 molecule^{-1} s^{-1}). The conjugate base of arginine is the sole exception as it undergoes three H/D exchanges with both of these reagents ($k = (7.1 \pm 2.1) \times 10^{-11}$ and $(1.3 \pm 0.4) \times 10^{-12}$ cm^3 molecule^{-1} s^{-1}, respectively) (Figure 2). These findings suggest that the deuterium is incorporated into the guanidine side chain, but to address this issue further, several methylated derivatives and α-desamino arginine ($H_2NC(=NH)NH(CH_2)_4CO_2^-$) were examined. The latter compound and *N*(α),*N*(α)-dimethylarginine ($H_2NC(=NH)NHCH_2CH_2CH_2CH(NMe_2)CO_2^-$) both incorporate up to three deuterium atoms with $C_6H_5CH_2OD$ ($k = (1.5 \pm 0.5) \times 10^{-10}$ and $(1.0 \pm 0.3) \times 10^{-10}$ cm^3 molecule^{-1} s^{-1}, respectively), whereas *N*(γ),*N*(γ)-dimethylarginine ($Me_2NC(=NH)NH(CH_2)_3CH(NH_2)CO_2^-$) does not react at all. These results confirm that three of the four hydrogens on the guanidine side chain undergo exchange, and they indicate that at least one hydrogen on the side-chain NH_2 group is required for the reaction to occur. A pathway which is consistent with these findings is the six-centered flip-flop mechanism illustrated in Scheme 1 in which the carboxylate presumably facilitates the process by hydrogen bonding to one of the sites on the guanidine side chain. Processes of this sort were previously proposed by Beauchamp et al.,[3] and other viable options are difficult to envision given the weak acidities of the exchange reagents that were employed.

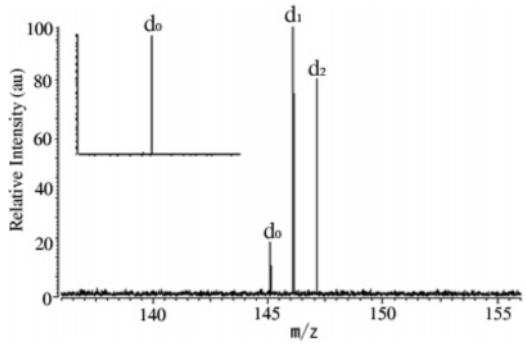


Figure 1. Mass spectra for the H/D exchange of deprotonated lysine with CF_3CH_2OD (2.4×10^{-7} Torr) at time 0.0 s (inset) and 28.5 s.

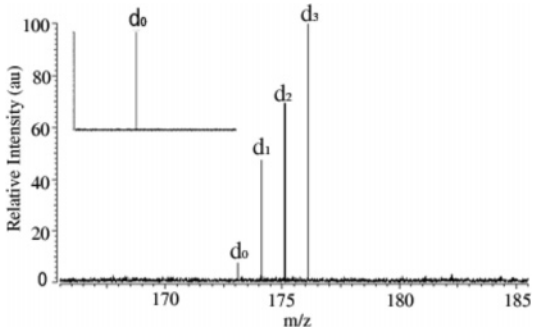
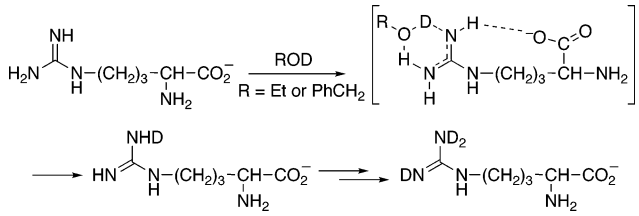


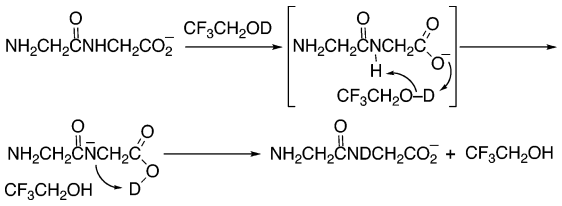
Figure 2. Mass spectra for the H/D exchange of deprotonated arginine with $C_6H_5CH_2OD$ (2.7×10^{-7} Torr) at time 0.0 s (inset) and 75 s.

10.1021/ja076599l CCC: $40.75

Scheme 1. Proposed Six-Centered Flip-Flop H/D Exchange Mechanism for the Incorporation of Three Deuteriums into the Side Chain of Deprotonated Arginine



Scheme 2. A Plausible Relay Mechanism for the H/D Exchange Reaction of the Gly-Gly M − H Ion with CF_3CH_2OD



The above findings are significant in that they indicate some anions can be differentially labeled in the gas phase even when this cannot be done in solution. Moreover, these results are not limited just to deprotonated amino acids. We have examined the H/D exchange of 24 dipeptide M − H ions with CF_3CH_2OD (i.e., Ala-Met (1), Ala-Phe (1), Ala-Ser (2), Ala-Trp (3), Ala-Val (1), *Arg-Tyr* (8), Arg-Val (4), *γ-Glu-Gly* (4), Gly-Glu (2), Gly−Gly (1), Gly-His (2), Gly-Phe (1), Gly-Pro (0), His-Gly (2), *His-Leu* (4), Leu−Leu (1), Lys-Phe (3), Lys-Ser (4), Met-Trp (1), Phe-Ala (1), Phe-Tyr (1), *Pro-Gly* (2), Pro-Leu (1), and Tyr-Arg (6)), where the number in parentheses indicates the number of exchanges that were observed. Only 1 deuterium is incorporated in 10 of the dipeptides even though they have 2−4 labile hydrogens. The amide hydrogen almost assuredly is the one being replaced in all of these substrates given that the reaction rates are within a factor of 3 of each other (i.e., $k = (5.7 \pm 1.7) \times 10^{-11}$ to $(1.6 \pm 0.5) \times 10^{-10}$ cm^3 $molecule^{-1}$ s^{-1}), and the one dipeptide that does not have an amide hydrogen (Gly-Pro) does not react (i.e., $k \leq 1 \times 10^{-13}$ cm^3 $molecule^{-1}$ s^{-1}). This leads us to suggest a relay process[3−5] for this transformation (Scheme 2) particularly since $\Delta\Delta H°_{acid}$ ($CH_3CONH_2 - CH_3CO_2H$) = 13 ± 3 kcal mol^{-1} and model calculations indicate that the difference in acidity between the amide and the carboxy group in Gly-Gly is only ∼8 kcal mol^{-1}.[8]

All of the hydrogens attached to oxygen and nitrogen undergo exchange in the four italicized dipeptides, and an intermediary number are replaced in the remaining nine dipeptides. It is unclear what controls this behavior, but the N-terminus generally is unreactive in the absence of an acidic or basic side chain. Our results also indicate that the order of the amino acids impacts the extent to which H/D exchange takes place. For example, Gly-Glu and Pro-Gly incorporate up to two deuteriums, whereas all four of the labile hydrogens undergo exchange in *γ*-Glu-Gly and Gly-Pro does not exchange at all. The unusual behavior of Lys and Arg also appears to be maintained in these dipeptides. More specifically, just one of the two amino groups in Lys-Phe and Lys-Ser undergoes exchange with CF_3CH_2OD. Likewise, four of the seven labile hydrogens in Arg-Val can be replaced with deuterium, which suggests that the amide hydrogen and three of the four hydrogens on the guanidine side chain undergo exchange. Substituted derivatives, however, should be used to confirm the locations of the reactive sites.

Several larger peptides have been reacted with CF_3CH_2OD, as well, and they too display a wide range of behavior. For example, all four N−H hydrogens undergo exchange in the M − 1 ion of Ala-Leu-Gly, but the amide sites react approximately 1 order of magnitude more rapidly than the N-terminus. In contrast, just three of the seven labile hydrogens are replaced with deuterium in Trp-Met-Asp-Phe, and $(Gly)_6$ does not react at all (i.e., $k \leq 1 \times 10^{-13}$ cm^3 $molecule^{-1}$ s^{-1}).

These results indicate that some small deprotonated peptide ions can be differentially labeled, which has not been demonstrated previously.[9,10] The extent of H/D exchange depends upon the primary sequence, both in terms of which amino acids are present and their order in the peptide chain. These findings raise many new questions but should be useful in carrying out mechanistic studies and addressing the mobile proton controversy.[11]

Acknowledgment. Support from the National Science Foundation and the Minnesota Supercomputer Institute is gratefully acknowledged.

Supporting Information Available: Table S1, Figure S1, the preparation of *N*(α),*N*(α)-dimethylarginine, the purification of α-desamino arginine and their NMR spectra along with a brief description of the gas-phase experiments are provided in conjunction with sample data (Figures S2 and S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) Incorporation of an additional deuterium was not observed under any of the conditions that were employed and has a rate constant of less than 2 × 10^{-13} cm^3 $molecule^{-1}$ s^{-1}. That is, it occurs at least 2 orders of magnitude or more slowly than the observed exchanges.

(7) The reported rates are the average of three measurements and have uncertainties which are assumed to be ±30%.

(8) For gas-phase acidities, see: Bartmess, J. E. *NIST Chemistry WebBook, NIST Standard Reference Database Number 69*; Mallard, W. G., Linstrom, P. J., Eds.; National Institute of Standards and Technology: Gaithersburg, MD 20899 (http://webbook.nist.gov).

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JA076599L